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THE ORIGIN AND NATURE OF THE SOIL ORGANIC NITROGEN

PART 1. AMINO ACIDS

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ABSTRACT

Approximately 10-12% of the total soil-N which occurs in the hydrolysable unidentified nitrogen fraction following the fractionation of soil hydrolysates by the Bremner method can be ascribed to amino acids. Revised estimates suggest that the total amino-N content of soils is approximately 50% of the total soil-N. Evidence is presented which suggests that the bulk of the soil organic nitrogen occurs in protein complexes. A large proportion of the nitrogen in these complexes, which can be formed *in vivo* and *post mortem* in Nature, becomes converted to acid soluble and insoluble forms during hydrolysis. A proportion of the nitrogen in these latter forms is probably the result of acid catalysed artifact formation between the complex moieties.

INTRODUCTION

Although various investigators have used different methods to study amino acids in acid soil hydrolysates, their results indicate that approximately 40% of the total soil nitrogen (total-N) occurs in amino acids (Morrow and Gortner 1917, Sowden 1977 Sowden *et al.* 1977) and that a significant proportion of the total N about 40% remains unidentified. The amount of total-N present as amino acids may be determined in neutralized hydrolysates by the commonly used method of Bremner (1965) (Bremner's (1965) hydrolysis and fractionation procedures hereafter called Bremner's method). Alternatively amino acids may be determined in desalted hydrolysates by ion-exchange chromatography (Stevenson 1965) (hereafter referred to as the ion exchange method).

In Bremner's method amino acids react with ninhydrin the α-amino-N is converted to NH_4-N and this is subsequently distilled off with alkali, collected in boric acid and determined by acid titration. Here separation of individual amino acids is not possible. In the ion exchange method the amounts of separated amino acids are determined spectrophotometrically after reaction with ninhydrin. Ninhydrin reacts with the α-amino-N group and hydrolysates of soil and litter contain a) amino acids which possess both non α-amino-N groups and α-amino-N (e.g. the basic amino acids) b) imino acids (e.g. proline and hydroxyproline)

c) amino acids with a single N atom not in the α configuration (e.g. α-amino-n-butyric acid and β-alanine). Since the ion exchange method can determine the amounts of individual amino acids including proline, it is possible to determine the total amino-N in a soil or litter. The Bremner method only gives an estimate of the total α-amino-N. In addition to a, b, and c above quantitative recovery of the α-amino-N in cystine is not achived although very small amounts of this amino acid may occur in soil and litter hydrolysates.

Overall, one would expect the total α-amino-N value of a soil whether determined by the Bremner or ion-exchange methods to be similar. Furthermore, from the results of the ion-exchange method it is possible to calculate the amino-N which is not estimated by the Bremner method. This value together with the total α-amino-N value would be expected to give a more accurate value for the total amino-N value of a soil or litter. If this is so then the results, commonly expressed as"amino-N" in the literature but which more correctly refer to partial α-amino-N of soils, obtained with the Bremner method are gross underestimates of the real values and since this is a widely used method published estimates for this form of N should be revised. An objective of the work was to ascertain by how much existing Bremner determined amino values should be increased. A further objective was to determine the degree of accuracy and reproducibility obtained by different investigators using either the Bremner or ion-exchange methods to determine the partial α amino-N or total amino-N in one soil and one litter sample.

MATERIALS AND METHODS

The soil, a clay loam (pH 7.2,0.44% N) originated from a mull site in Bagley Wood near Oxford (U.K.). The predominant tree species at this site was ash (*Fraxinus excelsior* L.) with a few scattered oak (*Quercus robur* L.). The surface layer (0-250 mm) of the soil was sampled in November 1969 by taking 80 random soil cores 40 mm. diam. The cores were combined, air dried, ground in a pot mill to a fine powder and the soil stored in air tight amber glass jars.

The little sample (1.25%N) consisted of a mixture of equal proportions (air dried weight) of freshly fallen litter of *Ulmus* sp., *Cupressus* sp., *Castanea* sp., *Acer* sp., and *Calluna* sp. The mixture was ground to a fine powder and stored in air tight amber glass jars.

The hydrolysis and fractionation of samples was performed exactly as specified by Bremner. The reflux temperature, not specified by Bremner, was 110°C and lasted 24 h except in the case of investigator F who used a 12 h hydrolysis period at 110°C. Workers using this method were investigators A and B, Europe, investigators C,D,E and F, New Zealand.

Investigators using ion exchange methods to analyse samples invariably differed amongst themselves and with those using the Bremner method as to the amounts of material and volumes of acid which were hydrolysed, and also in regard to the temperature and

and time of hydrolysis. In view of this, brief experimental details of each investigator as far as it has been possible to ascertain them, are given below. Results were reported in such a variety of ways (e.g. M sample wt^{-1}, µg sample wt $^{-1}$) that it was decided to convert all results to a mg g^{-1} sample basis.

Investigator G, Europe: 4.0g soil or 0.5 litter hydrolysed in sealed tubes rotated for 16 h at 115°C in an oven. Reproducibility between respective samples was good.

Investigator H, Europe: 0.5 g soil or 0.1 litter hydrolysed under reflux for 6 h at 120°C in 15 ml 6N HCl. Reproducibility between respective samples was good.

Investigator I, North America: 13.929 mg or 25.544 mg soil hydrolysed in vacuo for 20 h at 110°C with 0.28 ml or 0.5 ml 6N HCl respectively. Reproducibility between respective samples was good.

Investigator J, New Zealand: 97 mg soil or 70 mg litter hydrolysed under reflux for 22 h at 110°C with 50 ml 6N HCl. Only one determination of each sample performed.

Investigator K, S.E. Asia: 0.2 g soil hydrolysed in vacuo for 24 h at 110°C with 2 ml 6N HCl. Reproducibility between samples was good.

Investigator L, S.E. Asia: 2.0 g soil hydrolysed under reflux for 20 h at 110°C with 20 ml 6N HCl. Reproducibility between samples was good.

Investigator M, North America: 2.0 g soil hydrolysed under reflux for 24 h at 110°C with 60 ml 6N HCl. One determination only.

Investigator N, Europe: 2.0 g soil or 0.5 g litter hydrolysed under reflux 12 h at 110°C with 50 ml 6N HCl. Reproducibility between samples was good.

Investigator O, New Zealand: 0.1 g soil or 0.1 g litter hydrolysed in vacuo for 20 h at 100°C with 5 ml 6N HCl. Reproducibility between soil samples was good. One litter sample analysed.

Investigator P, Europe: 0.5 g soil or 0.5 g litter hydrolysed under reflux in a nitrogen atmosphere for 12 h at 100°C with 12 ml 6N HCl. Reproducibility between samples was good.

With the exception of investigator I who dried hydrolysates *in vacuo* over KOH and P_2O_5, all investigators removed excess HCl from hydrolysates by rotary evaporation at 40-50°C and subsequently applied buffered aliquots of the hydrolysates to ion-exchange columns.

During acid hydrolysis NH_4-N is formed due to the decomposition of hexosamines. The values reported for hexosamine-N have been adjusted by the use of correction factors and

NH_4-H values correspondingly adjusted. The correction factors have been determined for mixtures of soil, litter and pure hexosamines (chitin and glucosamine) hydrolysed in 6N HCl and were found to be 1.4 (24 h, 100°C); 1.4 (12 h, 110°C); 2.0 (24 h, 110°C); 1.4 (6 h, 120°C); 1.7 (12 h, 120°C) and 2.7 (24 h, 120°C). All results are reported as % total soil or litter N (Kjeldahl).

RESULTS AND DISCUSSION

The distribution of N obtained by various investigators for the soil and litter samples using the Bremner method are presented in Table 1. The results for the α-amino-N values and unidentified -N (hydrolysable unidentified -N+ acid insoluble -N) are similar for the respective soil and litter samples. Quantitative recovery of standard NH_4-N+ hexosamine -N+amino-N solutions were reported by all investigators.

TABLE 1. NITROGEN DISTRIBUTION ANALYSIS OF SOIL AND LITTER SAMPLES (BREMNER METHOD). RESULTS REPORTED AS % TOTAL SOIL OR LITTER N. HUN = HYDROLYSABLE UNIDENTIFIED NITROGEN.

Investigator		NH_4-N	hexosamine-N	α amino-N	HUN	insoluble N
Author	Soil	17	8	37	24	14
	Litter	10	0	46	26	18
A	Soil	17	8	36	25	14
	Litter	10	0	47	25	18
B	Soil	18	8	36	24	14
	Litter	11	0	46	24	19
C	Soil	17	8	36	25	14
	Litter	10	0	46	17	17
D	Soil	19	8	37	23	13
	Litter	11	0	47	25	17
E	Soil	19	8	36	24	13
F *	Soil	17	8	36	25	14

*one sample analysed

The amounts of amino acids in the samples determined by the ion-exchange method are presented in Table 2 from which the total amino-N values and theoretical Bremner α-amino-N values have been calculated. These latter values can be compared with the experimentally determined Bremner α-amino-N values given in Table 1.

A most striking result is the marked discrepancy between total amino-N values for soil or litter obtained by different investigators who used the ion exchange method. The exact causes of these discrepancies are probably many and varied although some general comments may be made. Although unpalatable it must be recognised that unsatisfactory results often arise from unsound scientific practices. Several investigators failed to check their calculations carefully and gave insufficient experimental conditions,

with little or no indication as to the recovery rates of standards. Many of the results in Table 2 therefore must be discounted, including those of investigator I who, although giving extensive experimental conditions and recovery rates, used smaller samples than is normally the case in soil nitrogen research.

Allowing for these discrepancies, one objective of this study is theoretically indicated: the experimental total amino-N values (ion-exchange method) are greater than the theoretical Bremner α-amino-N values. The results of investigators G, H, K and L deserve further examination for with one exception (investigator H, soil sample) the experimentally determined Bremner α-amino-N values (Table 1) are similar to those Bremner α-amino-N values derived from ion exchange data (Table 2). The discrepancy in the case of investigator H is probably due to the shorter hydrolysis time used (6 h). When samples of the soil and litter were subjected to similar hydrolysis conditions and the hydrolysates subjected to the Bremner method, then the experimental Bremner α-amino-N values were similar to those calculated in Table 2: 30% and 46% for soil and litter respectively. It seems likely that with this soil a 6 h hydrolysis period is insufficient to allow complete hydrolysis of nitrogenous substances complexed in inorganic aggregates. The litter sample with little inorganic material was completely hydrolysed at 6 h and 120°C. On the other hand litter hydrolysed for 6h at 100°C and analysed by the Bremner method gave α-amino-N values considerably lower than those obtained using 6 h at 120°C.

Extensive tests in the author's laboratory (unpublished but available for inspection on request) indicate that for substances such as leaf litter, animal and microbial tissues which contain little inorganic material or which are not complexed with large amounts of such material, a minimum hydrolysis time of 6 h at 120°C is necessary for complete hydrolysis and for routine analyses 12 h at 110°C is recommended. It is generally accepted that no two soils are completely alike in their physical, chemical and biological properties and tests have indicated that for a particular soil the optimum hydrolysis conditions need to be determined by trial and error. Soil organic and inorganic components are so tightly complexed that short hydrolysis periods at high temperatures have been found insufficient for complete release and hydrolysis of the organic component. In general for the routine nitrogen distribution analysis of soils a minimum time of 12 h hydrolysis at 120°C has been found necessary to give satisfactory results.

It is surprising to learn from the literature that the majority of investigators do not state their hydrolysis temperatures. With a few measurements one can deduce these temperatures. For example Sowden (1977) commonly applied a correction factor of 2 to his hexosamine-N values determined after a 24 h reflux hydrolysis. From studies using purified hexosamines this factor corresponds to a 24 h hydrolysis period at 110°C. Bremner (1965) in describing the details of his method suggests that soil-acid mixtures be gently boiled under reflux for 12 h. Tests show that gentle boiling of such mixtures occurs at a temperature of 117°C. It is important to state the precise temperature and time of a hydrolysis for another reason. The destruction of soil hexosamines and probably other forms of nitrogen in 6N HCl varies markedly according to the temperature and time of hydrolysis.

TABLE 2. AMINO ACIDS (mg $^{-1}$SAMPLE) DETERMINED BY THE ION EXCHANGE METHOD FOR SOIL (S) AND LITTER (L), tr = TRACE, NR = NOT REPORTED.
EXPERIMENTALLY DETERMINED BREMNER α AMINO-N VALUES FOR SOIL = 37%, LITTER 46% (TABLE 1)

	INVESTIGATOR															
	G		H		I	J		K	L	M	N		O		P	
amino acids	S	L	S	L	S	S	L	S	S	S	S	L	S	L	S	L
cysteic acid	0.11	1.17	NR	NR	NR	NR	NR	0	0	0	tr	tr	0	0	0	0
aspartic acid	2.39	5.32	1.87	5.27	2.05	2.9	7.3	2.37	2.43	2.39	2.05	5.43	3.05	7.12	2.75	7.48
threonine	1.01	2.89	0.83	3.4	0.73	1.13	3.46	1.13	1.13	0.91	0.90	3.14	1.17	3.49	1.09	3.44
serine	0.91	3.46	0.95	3.75	0.64	1.2	3.82	1.07	1.05	0.81	0.82	3.38	1.35	3.73	1.05	3.92
glutamic acid	1.71	6.69	1.28	5.99	1.36	2.03	7.49	1.81	2.11	1.63	1.47	5.95	2.11	6.94	1.96	8.05
proline	0.91	3.25	0.86	3.86	0.61	1.11	3.66	0.86	0.86	0.83	0.74	3.11	1.09	3.75	1.13	3.57
glycine	1.36	3.29	1.07	3.59	0.86	1.78	4.17	1.51	1.52	1.20	1.00	2.96	2.05	4.21	1.51	4.13
alanine	1.09	3.42	0.94	3.63	0.70	1.49	3.88	1.00	1.02	0.98	0.93	3.31	1.71	3.71	1.24	4.06
valine	0.95	3.49	0.64	3.09	0.77	0.97	3.22	1.06	1.06	0.84	0.67	2.57	1.09	3.07	0.86	3.38
cysteine	0.15	tr	0	0	0.07	0.18	0.35	0	0.04	NR	0.18	0.86	tr	tr	0.37	0.70
methionine	tr	tr	NR	tr	0.14	0.11	0.77	0.22	0.26	0.08	0.16	0.69	0.21	0.93	tr	0.62
isoleucine	0.66	2.88	0.66	2.72	0.41	0.61	2.57	0.45	0.42	0.53	0.45	2.17	0.72	2.36	0.51	2.78
leucine	1.01	5.04	0.96	6.27	0.68	0.61	5.34	1.12	1.12	0.85	0.78	4.30	1.2	5.32	1.02	5.36
tyrosine	1.39	2.56	0.31	1.55	0.20	0.82	2.96	0.17	0.21	0.12	1.88	2.69	0.64	3.15	1.00	2.77
phenylalanine	1.14	3.37	0.55	2.59	0.43	0.95	3.41	0.52	0.58	0.64	0.49	2.82	1.03	3.42	0.87	3.41
lysine	0.85	2.98	0.87	3.29	0.51	1.78	3.51	0.73	0.95	0.71	0.64	2.42	2.63	3.34	0.93	3.68
histidine	0.35	1.17	0.18	0.85	0.19	0.57	1.42	0.32	0.33	0.30	0.24	1.04	0.68	1.38	0.46	1.47
arginine	0.61	3.14	0.52	3.12	0.42	1.01	3.32	0.54	0.67	0.54	0.43	2.35	1.1	3.18	0.92	3.47
Total amino-N (% total N)	49	61	38	61	32	64	69	46	49	41	40	55	72	67	55	71
theoretical Bremner α-amino-N (% total N)	40	47	30	46	26	49	53	37	39	33	33	43	54	52	43	55

Extensive tests in this laboratory and reported in the literature (Bremner 1949, Sowden 1969, Roach and Gehrke 1970, Gottschalk 1972, Davies and Thomas 1973, Savoy *et al.* 1975) indicate no significant alteration of the nitrogen distribution analyses of a variety of soils, litters and proteins using open and closed hydrolysis systems and analysis by the Bremner or ion-exchange methods (although a large acid vol. : sample wt ratio appears to be important). With care and experience, negligible amounts of nitrogen are lost during the filtration and washing stage of acid hydrolysed materials, although the filtered residues must be well washed to remove occluded nitrogen. Rotary evaporation or vacuum desiccation of filtered acid hydrolysates has also been shown not to be a major source of nitrogen loss. Bremner devised a beautifully simple method for determining the nitrogen distribution analysis of a soil and there is no evidence to suggest that serious error may result from interference by inorganic or organic substances during the neutralisation procedure, or during the ninhydrin reaction in that method. It is still not an easy task to separate, elute and estimate quantitatively all the amino acids present in soil and litter hydrolysates from resin columns because of the interference caused mainly by substances containing Fe and Al. This interference if not removed at an earlier stage may persist to the stage when the amino acids are estimated. Furthermore, the presence of some organic substances produced during the acid hydrolysis procedure from carbohydrates (e.g. levulinic acid) may intensify this interference (Smith, 1965, Sowden 1969, Gottschalk 1972, Pigman and Horton 1972, Davies and Thomas 1973, Feather and Harris 1973, Taylor and Cameron 1973, Cheng 1975, Cameron and Taylor 1976). It appears then that many details need to be scrupulously observed, notwithstanding those factors as yet imprecisely understood, when acid hydrolysates of soils or litters are subjected to analysis by ion exchange chromatography. In contrast, the Bremner method can be performed by persons with little prior expertise and there is no evidence which suggests that the values reported for the NH_4-N, hexosamine-N and α-amino-N factions are wholly or partially the result of interference effects.

Many of the problems which beset the accurate determination of amino acids occurring in soil hydrolysates by ion-exchange methods are appreciated to a greater extent in those laboratries engaged in aspects of soil nitrogen research. This was the case for the results in Table 2 reported by investigators G, H, K and L where it is clear that the Bremner α-amino-N values calculated from the results of ion-exchange analysis show considerable agreement with the values directly determined (Table 1) for this fraction. Thus there is evidence which supports the suggestion that the α-amino-N values reported for a wide range of soils and litters using the Bremner method are underestimates and that the Bremner hydrolysable unidentified nitrogen (HUN) fraction contains a considerable amount of nitrogen derived from amino acids. This being the case, these findings support the result of an earlier study (Greenfield 1972) which suggested, based on evidence from analysis of pure proteins by the Bremner method together with a consideration of Handley's (1954, 1961) views, that a considerable proportion of the total nitrogen occurring in unfractionated soils and litters occurs in the form of protein.

To check further the accuracy of the Bremner method, investigators H, G and L who had used the ion-exchange method and investigators A B and F who had used the Bremner method were requested to submit soil samples for analysis by the author using the Bremner method. The results of this survey are presented in Table 3 and it can be seen that the trends initiated in Table 2 are reinforced. The experimentally determined Bremner α-amino-N values for soils H1, H2, G1, G2 and L1 are consistently 10-12% (total soil - N basis) lower than the total amino - N values determined by ion-exchange methods. In the case of the Bremner α-amino-N values for soils A1, B1, B2 and F1 considerable agreement exists between the values determined by the author and investigators A, B and F.

TABLE 3. COMPARISON OF TOTAL AMINO-N (ION-EXCHANGE) AND α-AMINO-N (BREMNER METHOD) VALUES OF SUBMITTED SOIL SAMPLES WITH α-AMINO-N VALUES DETERMINED BY THE AUTHOR USING THE BREMNER METHOD. RESULTS EXPRESSED AS % OF TOTAL SOIL N.

Investigator	Soil	%N	total amino-N (ion exchange)	α-amino-N determined by the author (Bremner method)
H	Soil H1	0.14	51	37
	Soil H2	0.62	43	33
G	Soil G1	0.29	48	33
	Soil G2	0.17	47	36
L	Soil L1	0.42	38	26
			α-amino-N (Bremner method)	
A	Soil A1	0.58	32	33
B	Soil B1	0.11	40	40
	Soil B2	0.24	41	41
F	Soil F1	0.16	33	31

The evidence so far presented supports the view that a significant proportion of the soil nitrogen occurs in the form of amino acids. It also suggests that more research comparing the N distribution analyses of soils using both ion-exchange and Bremner methods is necessary particularly in soil laboratories with ion exchange facilities in the form of automatic amino acid analysers. Indeed, with further improvements in separation techniques, the ion-exchange method may be capable of resolving more of the remaining unidentified nitrogen into amino acids.

A further check on this was made in the following manner. Samples of the soil and litter were hydrolysed and filtered (Materials and Methods). Excess HCl was removed on a rotary evaporator at 40°C and each concentrated hydrolysate made up to 50 ml and shaken well. Twenty-five ml of each solution were withdrawn, neutralised and factionated according to the Bremner method. The remaining 25 ml of each solution were delivered to investigator J for analysis by ion exchange chromatography within three hours. The procedure was repeated and subsamples sent by airmail to investigator L. The results are presented in Table 4 which shows that when soil and litter hydrolysate

subsamples were subjected to both the ion exchange and Bremner methods the total amino-N values (ion-exchange method) were substantially greater than the Bremner α-amino-N values (Bremner method) by approximately 12% of the total-N in either soil or litter. Consequently the HUN values determined by the Bremner method are 12% or so higher than those determined by the ion exchange method. Although this view was deduced by Greenfield (1972) experimental proof has been lacking until now. This view is further strengthened in the light of the present investigation by inspection of the nitrogen distribution analyses for various soil and litter hydrolysates (Sowden 1959, Sowden and Ivarson 1974, Sowden 1976, 1977, determined by the ion exchange method were the unidentified -N (HUN + insoluble-N) represents approximately 30% of the total soil or litter N. Approximately 50-60% of the total N in surface soils and litters is contained in the form of amino acids. Such high values argue in favour of this nitrogen occurring in the form of protein.

TABLE 4. TOTAL AMINO-N (ION EXCHANGE) AND α AMINO-N (BREMNER METHOD) VALUES DETERMINED ON SUBSAMPLES OF LITTER AND SOIL HYDROLYSATES. RESULTS EXPRESSED AS % OF TOTAL SOIL OR LITTER N.

Investigator		total amino-N (ion exchange)	α-amino-N determined by the author (Bremner method)
J	Soil	49	37
	Litter	58	46
L	Soil	48	37
	Litter	60	46

GENERAL DISCUSSION

Nitrogen distribution analysis of soils and litters reveals little about the origin and nature of the nitrogenous substances in the unfractionated soils and litters. The methods of nitrogen distribution analysis do allow however, qualitative and quantitative comparisons between different soils and litters.

Recent work (Sowden *et al.* 1977) suggests that for a wide range of soils under different climatic conditions the N distribution analyses are similar.

In addition to the commonly isolated amino acids such as those indicated in Table 2, chromatographic methods (paper and ion exchange) have detected, and in some cases quantified, a large array of amino acids which occur in small amounts in soil and litter hydrolysates (Sowden 1977, Bremner 1967). Such amino acids were probably present in the soil sample used in this study. In general, the types of amino acids detected in a wide variety of soils have been found to be similar, although the amounts of a particular amino acid may vary between soils (Stevenson 1954, 1956, Young and Mortenson 1958, Carles and Decau 1960, Wang *et al.* 1967, Almeida *et al.* 1969, Decau 1969).

Some of the amino acids detected in soil or litter hydrolysates have probably arisen from the destruction of other amino acids which are partially or wholly unstable under the hydrolytic conditions. In view of the difficulties involved in the separation and detection of even the commonly occurring amino acids by chromatographic methods it is not surprising that the cumulative contribution to the total amino-N value of these amino acids known to exist in soils, albeit in small amounts, has received little attention in ion exchange chromatography. The nitrogen originating from this source appears to represent approximately 2-3% of the total soil nitrogen (Young and Mortensen 1958, Sowden 1976). It is worthwhile noting here that the amino acid tryptophan is destroyed during acid hydrolysis and that the bulk of the nitrogen contained therein is converted to a form insoluble in acid, probably by artifact formation with carbohydrate degradation products. Inspection of the reports concerning the amino acid composition for purified proteins derived from plants, animal and microbial tissues indicates that not only do they contain a significant amount of tryptophan, but also that the amount of amino-acid nitrogen expected to occur in the HUN fraction following hydrolysis and fractionation by the Bremner method would be considerable and of the order of 20% of the total protein nitrogen (Stokes and Gunness 1946, Freeland and Gale 1947, Lugg 1949, Smith and Agiza 1951, Kelly 1953, Fowden 1954, Block 1956, Bird 1957, Watson 1958, Mateles 1968, Siren *et al.* 1970, Dayhoff 1972, Croll 1976).

In Nature, the proteins of living systems are usually combined with other cellular constituents for structural or functional purposes e.g. chloroplasts, microbial cell walls, animal cuticles. Many of the proteins and substances such as the hexosamines which have a structural function are further protected against the physical, chemical and biological rigours of their environment by combination with non-nitrogenous substances such as carbohydrates and aromatic moieties such as occur in plant and microbial walls and animal cuticles. On the death of many plant, animal and microbial tissues the cellular nitrogenous materials, mainly proteins, become further complexed (covalently) with non-nitrogenous substances. Details of the nature of the substances and the reaction involved in the formation of these protein complexes *in vivo* and *post mortem* are outside the scope of this article but they essentially involve the rapid oxidative polymerization of simple and complex substances such as hydroxyphenols, catechin type compounds, tannins and their covalent linkage with amino and sulphydryl groups present in amino acids and proteins. Furthermore, the resulting tanned complexes may physically and perhaps chemically combine with other cell constituents, e.g. cell walls. Undoubtedly tanned complexes such as the residual protein complexes in dead plant tissues (Handley 1954, 1961, Babel 1975), and the melanins of microbial tissues especially cell walls (Bu'Lock 1961, Durrell 1964, Bloomfield and Alexander 1967, Nicolaus 1968, Bull 1970, Meyer 1970, Webley and Jones 1971), animal cuticles (Lee 1965, Okafor 1966, Wigglesworth 1972, Brown 1975, Croll 1976) are of widespread occurrence and whilst these various complexes are very resistant to enzymic attack, it seems likely that the nature of the substances involved and sequence of events leading to the tanning differs in different cases.

In view of the above there seems to be little value in considering the resistance to mobilization shown by the soil organic nitrogen by reference to those obscure artifacts produced during the extraction and fractionation of soil and litter with caustic soda, the so-called humic and fulvic acids, for there is an abundance of naturally occurring resistant nitrogenous complexes reaching (dead plant tissue) and occurring (dead plant roots, micro-organisms and soil fauna) in the soil. Indeed, as will be reported in a later communication, extraction and fractionation, using the conventional techniques, of naturally occurring materials containing these resistant complexes or model complexes prepared under the appropriate physiological conditions causes the production of humic, fulvic and humin fractions. These fractions are analogous to those obtained from whole soils and exhibit similar "physical, chemical and biological" characteristics.

The suggestion (Handley 1954, 1961) that the bulk of the soil organic nitrogen may be derived directly from dead plant tissues and under some conditions (e.g. raw humus) persist for a considerable period of time clearly deserves more attention. This suggestion can be slightly modified in the light of recent investigations, to include the contribution made to the soil organic nitrogen contained in the form of predominately dead microbial and animal tissues (e.g. cell walls and cuticles together with microbial metabolic by products). The resistance to biochemical decomposition demonstrated by the proteins in soils and leaf litters is considered to be a property resulting among other things from their chemical union with non nitrogenous substances (e,g, tannins and carbohydrates). It is probable that the different nitrogen mobilization capacities demonstrated by the contrasting soil systems, mull and mor, can be ascribed in the first instance to the differing biological systems operating on residual protein complexes in these soil systems. If we now re-examine the N-distribution analyses reported in Table 1 for the mull soil and for soils reported in the literature it is not difficult to realise that the N-fractions reported have originated largely as a result of the destruction, during hydrolysis, of nitrogen containing complexes such as those occurring in dead plant, microbial and animal tissues. It is likely that the proportions of nitrogen occurring in the fractions in soil hydrolysates will be determined largely by the hydrolysis conditions and the analytical methods and to a smaller extent by the physical and chemical properties of the soils.

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